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Welcome to The Genotyping Protocol System

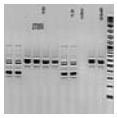
Master Protocol

Strain Name: C57BL/6NJ-*Prkab1*^{em1J}/J
Stock Number: 027203
Allele: Prkab1^{em1J}
Protocol Name: Prkab1^{em1J}
Method: Standard PCR
Created: 06-July -2015 (JKELMEN) **Updated:** 14-September-2015 (ESCHAAB)

Notes

Notes:	This allele from project Prkab1-6663J M#9043 was generated at The Jackson Laboratory by injecting Cas9 RNA and 3 guide sequences: CCTGTCCATCGAAACACGGT, AGAGGCGTTTACTTGGGGTCAGG, CCGAGATCCTTACCTTCTCG, which resulted in a 208 bp deletion in exon2 beginning at Chromosome 5 negative strand position 116021465 (CCCAAGTAAACGCCTCTGCTT) and ending after (AGGTAAGGATCTCGGCGGAC) at 116021672 bp (GRCm38/mm10). This mutation deletes exon2 and is predicted to cause amino acid sequence changes after residue 53 and early truncation 2 amino acids later.
Expected Results:	Mutant = 345 bp Heterozygote = 345 bp and 553 bp Wild type = 553 bp

Attachments



View 27203.jpg

\- View Prkab1em1j_final_6-26-15.docx

\- View Prkab1 genomic.gcs

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
22750	-	GCA CTG CAT GTG TCA GAC TGT	-	Forward	-
22751	-	GTC CAG GAG TCC GAT ACA GA	-	Reverse	-

Reaction/Components A

Reaction Components	Volume Amt	Final Concentration	Total Volume Amt
ddH2O	4.55	-	-
5 X Kapa 2G HS buffer	2.40	1	-
25 mM MgCl2	0.96	2	-
10 mM dNTPS-kapa	0.24	.2	-
20 uM 22750	0.30	.5	-
20 uM 22751	0.30	.5	-
5 mM 10x Loading Dye	1.20	.5	-

Cycling

Step #	Temp°C	Time	Note
1	94	2 min	-
2	94	20sec	-
3	65	15sec	-0.5 C per cycle decrease
4	68	10sec	-
5	-	-	repeat steps 2-4 for 10 cycles
6	94	15sec	-

Reaction/Components A			
2.5 U/ul Kapa 2G HS taq polymerase	0.05	.01	-
DNA	2.00	-	-

Cycling			
7	60	15sec	-
8	72	10sec	-
9	-	-	repeat steps 6-8 for 28 cycles
10	72	2 min	-
11	10	-	hold

Number Of Reactions 1

Version 3.2